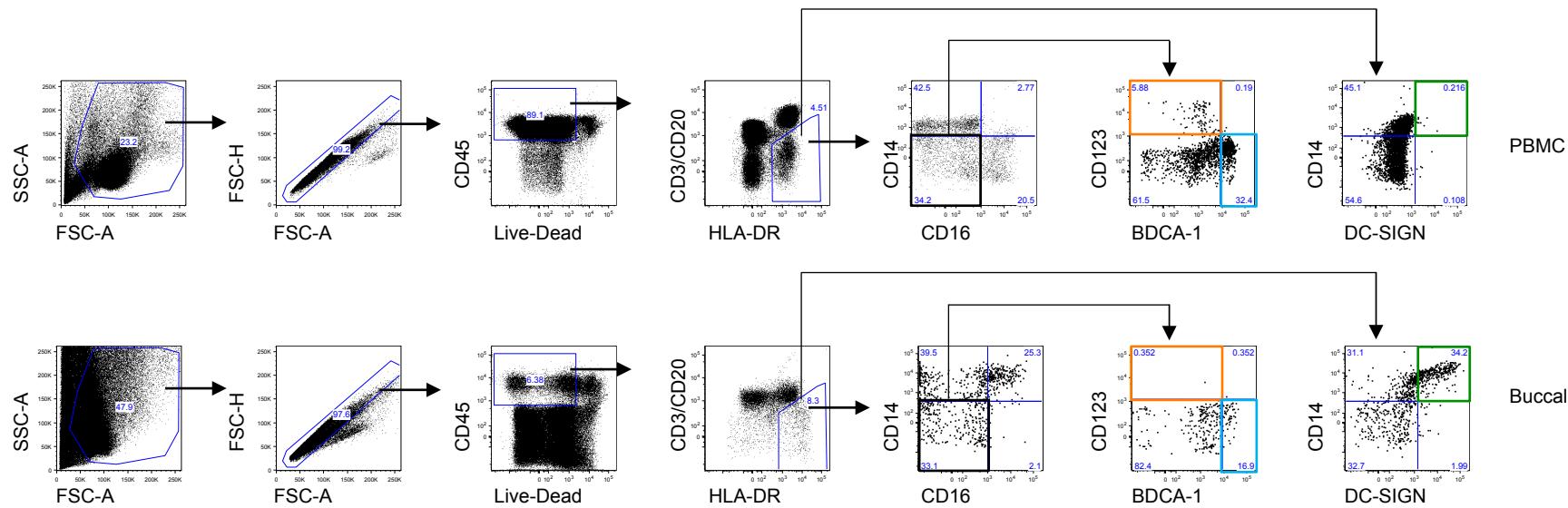
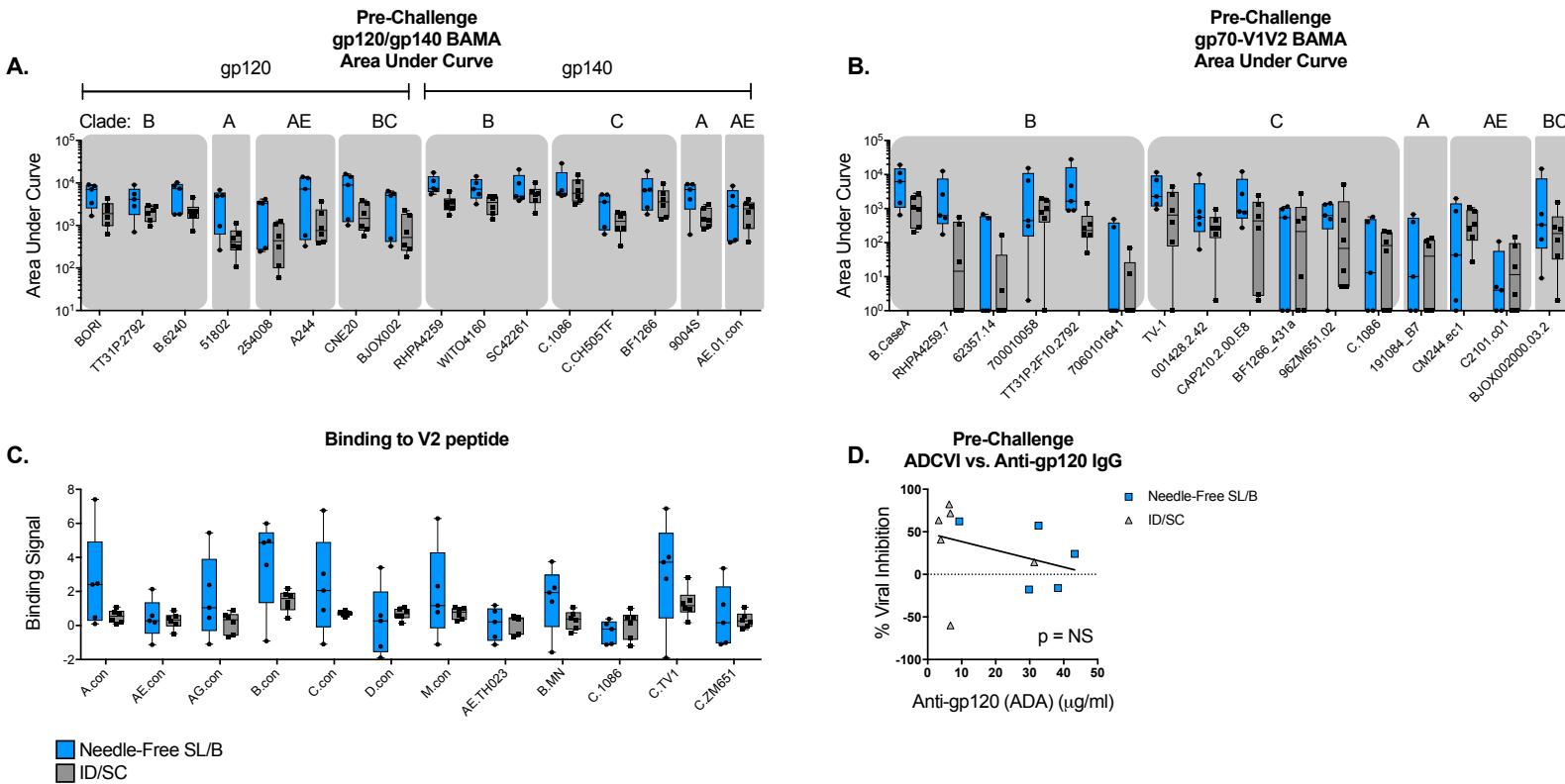


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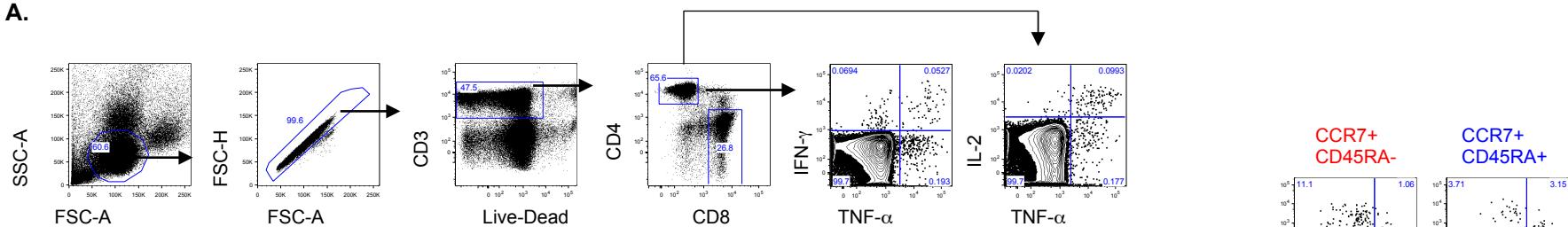
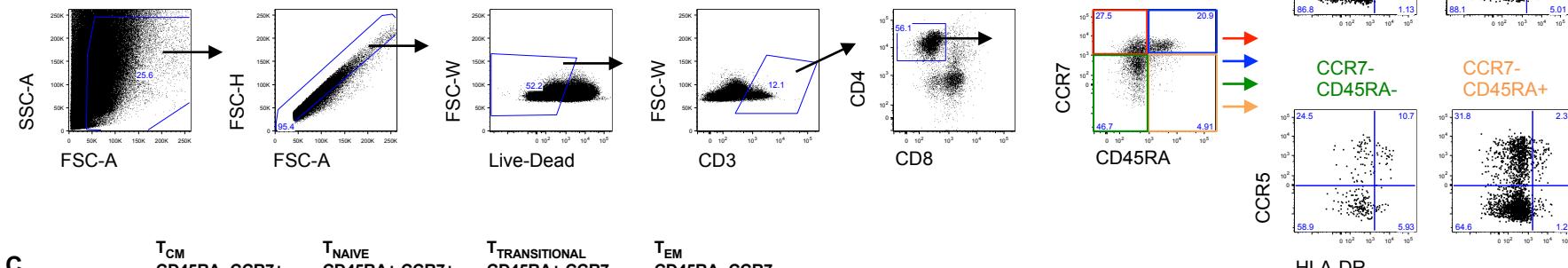
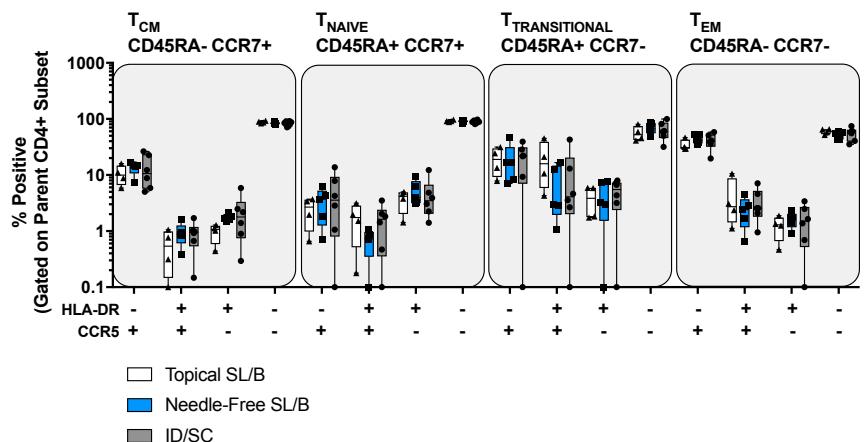
Supplementary Information



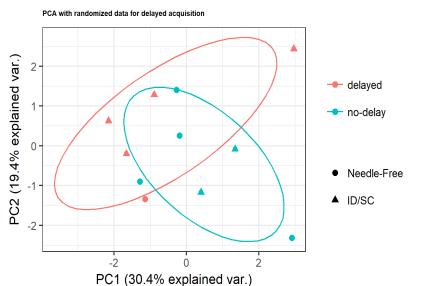
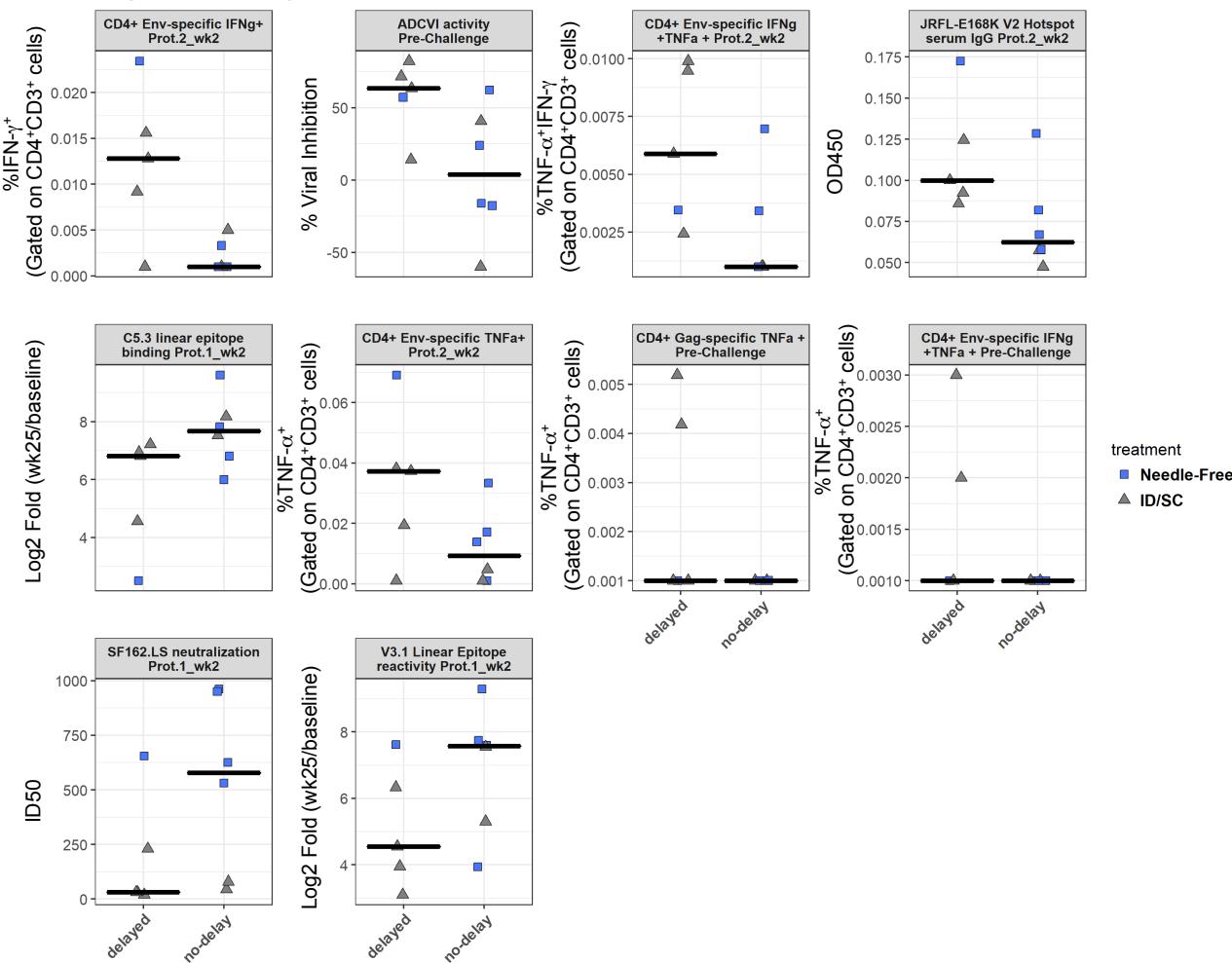
Supplementary Figure 1: Gating strategy for dendritic cells in PBMCs and tissues. Representative gating for conventional dendritic cells (CD45⁺ CD3⁻ CD20⁻ HLA-DR⁺ CD14⁻ CD16⁻ BDCA-1⁺ CD123⁻ Live cells), plasmacytoid dendritic cells (CD45⁺ CD3⁻ CD20⁻ HLA-DR⁺ CD14⁻ CD16⁻ BDCA-1⁻ CD123⁺ Live cells), and dermal dendritic cells (CD45⁺ CD3⁻ CD20⁻ HLA-DR⁺ CD14⁺ DC-SIGN⁺ Live cells) in PBMCs (top) and buccal tissue (bottom). Sublingual tissue, submandibular lymph nodes, submental lymph nodes, and inguinal lymph nodes are gated similarly. Blue gate, conventional DCs; orange gate, plasmacytoid DCs; green gate, dermal DCs.



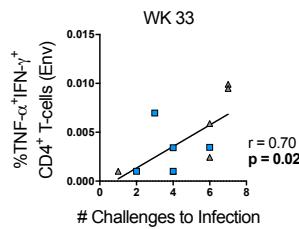
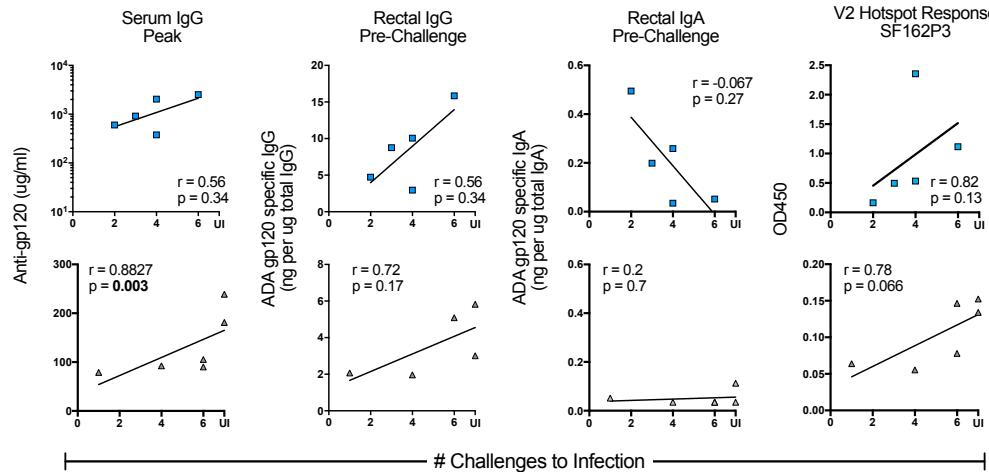
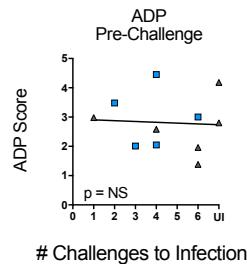
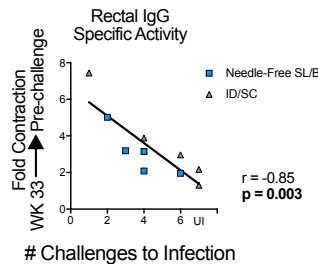
Supplementary Figure 2: Antibody magnitude, specificity, and effector functionality. BAMA analysis of ID/SC and needle-free SL/B immunized animal serum IgG at time of pre-challenge (wk 45) against gp120/gp140 antigens (A) and gp70-V1V2 antigens (B), quantified as Area Under Curve (AUC) analysis. HIV-1 Env strains organized by clade (B, C, A, AE, BC). (C) Serum IgG (wk 25) binding to linear V2 peptides from consensus clade A, AE, AG, B, C, D, group M, and viral strains AE.TH023, B.MN, 1086.C, C.TV-1, and C.ZM651, measured by peptide microarray linear epitope mapping and reported as Binding Signal (Log2 fold difference post-immunization/baseline binding intensity). (D) Correlation analysis comparing ADCVI activity to anti-gp120 IgG in serum at pre-challenge in needle-free SL/B (blue square) and ID/SC (grey triangle) immunized animals. Spearman correlation test. (A-C) Box and whiskers plot; box extends from 25th to 75th percentile, line indicates median, whiskers indicate min and max values. Blue shade, needle-free SL/B (n=5), grey shade, ID/SC (n=6).

A.**B.****C.**

Supplementary Figure 3: T-cell gating and phenotype. (A) Gating strategy for CD4⁺ and CD8⁺ T-cells (Live CD3⁺) cells in PBMCs, gated for IFN- γ , TNF- α , and IL-2 expression. Cytokine plots represent Env-peptide stimulated CD4⁺ T-cells from needle-free SL/B immunized animal (wk 25). (B) Gating strategy for rectal CD4⁺ T-cell subsets (Live CD3⁺CD4⁺). Central memory (T_{CM}) (CCR7⁺CD45RA⁻) (Red), Naïve (CCR7⁺CD45RA⁺) (Blue), Transitional memory (CCR7⁻CD45RA⁺) (Orange) and effector memory (T_{EM}) or effector (CCR7⁻CD45RA⁻) (Green) CD4⁺ subsets were gated for CCR5 and HLA-DR expression. (C) Frequencies of HLA-DR and CCR5 expressing CD4⁺ T-cell subsets in rectum, taken at the pre-challenge time point (wk 45). Box and whiskers plot; box extends from 25th to 75th percentile, line indicates median, whiskers indicate min and max values. White shade, Topical SL/B (n = 4), Blue shade, needle-free SL/B (n=5), grey shade, ID/SC (n=6).

A.**Randomized Data****B.****Delayed versus non-delayed infection for PC1 variables**

Supplementary Figure 4: Principal component analysis of immune parameters in delayed and non-delayed infected animals. (A) PCA performed as in Fig. 6d with randomized assay data. Animals separated into delayed (≥ 5 challenges to be infected) ($n=5$) vs. non-delayed (< 5 challenges to be infected) ($n=6$) groups. **(B)** Comparison of immune parameters included in the principal component 1 (PC1) of the PCA, separated by animals showing delayed or non-delayed SHIV-SF162P3 acquisition. Line indicates median. Blue square, needle-free SL/B; grey triangle, ID/SC

A.**B.****C.****D.**

Supplementary Figure 5: Correlates of protection against SHIV-SF162P3 infection. (A) Correlation analysis of %TNF- α ⁺IFN- γ ⁺ CD4 $^+$ T-cells in response to Env peptide stimulation two weeks post the second protein boost (wk 33) and acquisition of infection. Both groups, needle-free SL/B and ID/SC are combined ($n=11$). (B) Correlation analysis of peak serum (wk 25) gp120 specific serum IgG, gp120 specific activity of rectal IgG and IgA at the pre-challenge timepoint (wk 45), and peak SHIV-SF162P3 V2 hotspot responses (WK 25, Needle-free oral; WK 33, ID/SC) and acquisition of infection. Vaccine groups separated by needle-free SL/B (blue square, $n=5$) and ID/SC (grey triangle, $n = 6$). (C) Correlation analysis of ADP activity at pre-challenge and acquisition of infection. Groups combined. (D) Correlation analysis comparing the fold contraction of gp120-specific rectal IgG (specific activity) from two weeks post the second protein boost (wk 33) to the pre-challenge time point (wk 45) and acquisition of infection. Groups combined. (A-D) Spearman correlation analysis.

Epitope	Peptide Region	AA Range (HXB2 Nbr)
C1.1	#34-35	aa101-118
V2	#54-55	aa166-183
C2.1	#66-67	aa202-219
C2.2	#82-84	aa250-270
C2.3	#88-89	aa268-285
V3.1	#99-101	aa301-321
V3.2	#102-103	aa312-329
C3	#122-123	aa373-390
C4	#136-138	aa424-444
V5	#148-149	aa460-477
C5.1	#152-153	aa472-489
C5.2	#157-159	aa487-507
C5.3	#160-161	aa496-513

Supplementary Table 1: Linear epitope mapping peptide positions in HIV-1 reference strain HXB2.